MARKED UP VERSION TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Table 2 starting on page 26 was amended as follows:

Table 2

reagent	final concentration
KC1	50mM
Tris-HC1, pH 8.3	10mM
$MgCl_2$	3.5mM
dATP	200μM
dCTP	200μM
dGTP	200μM
dUTP	400μM
β-actin forward primer (SEQ ID NO:[Sequence No.]1)	300nM
β-actin reverse primer (SEQ ID NO:[Sequence No.]2)	300nM
β-actin probe (SEQ ID NO:[Sequence No.]3)	200nM
[AmpliTaq]AMPLITAQ DNA polymerase	0.25U/μ1
[AmpErase]AMPERASE UNG	0.01U/μ 1
human male DNA (Two types of samples, one containing human male DNA and the other containing no human male DNA, were prepared.)	0.2ng /μ1

Paragrah 2 on page 39 was amended as follows:

[Fig.32] Fig. 32 is an exploded view of the holder containing the reaction vessels incorporated therein. The reaction vessels 100 are fixedly fitted in concavities of the holder body 130. After the fitting of the reaction vessels 100 in the holder 130, a fixing plate 133 is detachably attached on to an upper surface of the body with an easily releasable adhesive, [Velcro]VELCRO or the like. The fixing plate 133 is made of a thin metal plate having excellent thermal conductivity and has a role to fix the reaction vessels to the holder body 130. To increase heat transfer between the holder body 130, the reaction vessels 100, and the

fixing plate 130, interstices there between are filled with oil or a grease. When placed in a thermal cycler, the holder is placed with the side of the fixing plate 133 in contact with a heat block.

Paragraph 2 on page 40 was amended as follows:

To prevent adsorption of an enzyme on an inner wall of an ink jet head, study was made on coating. As a system for optically detecting whether PCR proceeded or not, [Taqman System]TAQMAN SYSTEM (produced by Perkin Elmer Co.) was employed. A reaction solution for PCR was prepared as shown in the following ["Table 3".]"Table 3."

Table 3 starting on page 40 was amended as follows:

Table 3

Composition of Taqman PCR system

containing a human genome as a template

reagent	final concentration
KCl	50mM
Tris-HCl, pH 8.3	10mM
MgC12	3.5mM
dATP	0.2mM
dCTP	0.2mM
dGTP	0.2mM
[dTTP]dUTP	0.[2]4mM
β-actin forward primer (SEQ ID NO:[Sequence No.]1)	0.3μM
β-actin reverse primer (SEQ ID NO:[Sequence No.]2)	0.3μM
β-actin probe (SEQ ID NO:[Sequence No.]3)	0.2μM
[AmpliTaq]AMPLITAG DNA polymerase	0.1U/ μ L
[AmpErase]AMPERASE UNG	0.01U/ μ L
human male genomic DNA (produced by Boehringer Mannheim Co.)	lng/μ L

Table 5 on page 47 was amended as follows:

Table 5

reagent	final concentration
KC1	50mM
Tris-HC1, pH 8.3	10mM
MgC12	3.5mM
dATP	0.2mM
dCTP	0.2mM
dGTP	0.2mM
[dTTP]dUTD	0.4mM
β-actin forward primer (SEQ ID NO:[Sequence No.]1)	0.3 μ M
β-actin reverse primer (SEQ ID NO:[Sequence No.]2)	0.3 μ M
β-actin probe (SEQ ID NO:[Sequence No.]3)	0.2 μ M
[AmpliTaq]AMPLITAQ DNA polymerase (4-fold concentration relative to one for a reaction in a tube on a usual scale)	0.1U/μ L
[AmpErase]AMPERASE UNG	0.01U/μ L
target DNA (SEQ ID NO:[Sequence No.]4)	1ng/μ L

Table 7 starting on page 50 was amended as follows:

Table 7

reagent	final concentration
KC1	50mM
Tris-HC1, pH 8.3	10mM
$MgC1_2$	3.5mM
dATP	0.2mM
dCTP	0.2mM
dGTP	0.2mM
[dTTP]dUTD	0.4mM
β-actin forward primer (SEQ ID NO:[Sequence No.]1)	0.3 μ M
β-actin reverse primer (SEQ ID NO:[Sequence No.]2)	0.3 μ M

β-actin probe (SEQ ID NO:[Sequence No.]3)	0.2 μ M
(20-fold concentration relative to one for	
a reaction in a tube on a usual scale)	
[AmpErase]AMPERASE UNG	0.01U/μ L
target DNA (SEQ ID NO:[Sequence No.]4)	1ng/μ L

The first paragraph on page 51 was amended as follows:

A sample were subjected to reaction under a thermal cycle of at 50°C for [2min]2 min, at 95°C for [10min]10 min, and under 40 times repetition of a cycle of at 92°C for [lmin]1 min, at 54°C for [lmin]1 min and 72°C for [2min] 2 min, and further at 72°C for [10min]10 min, and observation with a fluorescence microscope was conducted. As a result of the observation, in comparison between the sample (+) subjected to the thermal cycle and a sample (-) unsubjected to the thermal cycle, change in fluorescence was clearly observed. The (+) sample showed a light green fluorescence and the (-) sample showed a reddish yellow fluorescence. The difference in fluorescence corresponded to the difference in fluorescence spectrum between a case where PCR proceeded and a case where PCR did not proceed when [Taqman]TAQMAN PCR was conducted in a usual tube. It is considered that PCR progressed in aqueous droplets in the (+) sample.

The second paragraph starting on page 51 was amended as follows:

The reaction vessels of (+) and (-) samples were superimposed, and aqueous droplets in both the reaction vessels were microphotographed (taken) in one photomicrograph. As a film, a color slide film ([Ektachrome]EKTACHROME DYNA EX, IS0100, produced by Kodak Co.) was used. The image in the developed film was captured into a personal computer with a film scanner (Quick scan 35, manufactured by Minolta Co.,Ltd.) and analyzed by means of an image analysis software ([Photo shop]PHOTO SHOP, produced by Adobe Systems Inc.). Arbitrary 30 points were taken (30 points were taken at random) in the fluorescence image of the aqueous droplets of the two samples, and color separation was conducted to obtain values of red (R), green (G) and blue (B). Ratios of R and G to R+G+B were plotted in [Fig.39]Fig. 39. It is seen that the (+) sample and the (-) sample emitted

evidently different fluorescences.

Paragraph 2 starting on page 52 was amended as follows:

7) Experimental Example

From the above-described studies, it was found to be advantageous that PCR be performed under the following conditions. Thereupon, PCR was performed under the conditions.

(a) use of a thin reaction vessel having an inside thickness of 50μm or less (which has a simple structure and is inexpensive) to prevent dissolution of a reaction solution in an oil

(b) vessel surface coatings capable of realizing both of prevention of adsorption of [oxygen] enzyme and prevention of scattering of aqueous droplets

(c) acceleration of PCR by increasing an amount of an [oxygen] enzyme

The second paragraph starting on page 53 was amended as follows:

After completion of the reaction, all portions of the solution were collected into one in order to remove the Primers and the like, and the solution was purified by ultrafiltration. The solution was divided into portions in 6 tubular ultrafiltration membranes ([Microcon] MICROCON 1000, produced by Amicon Co.) and filtered by a centrifugal separator at 500G for [24min.]24 min 300μ1 of TE buffer (pH 8.0) was added to each of the portions in the tubes, and the portions of the solution were further filtered by the centrifugal separator at 500G for 15 min and washed. The washing was conducted two times. 10μ1 of TE buffer (pH 8.0) was further added to each tube to dissolve the purified sample which remained on the ultrafiltration membrane. [Fig.40]Fig. 40 shows results of analysis by means of 0.8% agarose gel electrophoresis. Lanes in [Fig.40]Fig. 40 show results of electrophoretic migrations of the following respective samples.

lane 1: molecular weight marker ϕX174/HincII (2.6μg)

lane 2: pre-ulatrafiltration reaction solution (10μ1)

lane 3: post-washing solution (first washing) (10μ1)

lane 4: post-washing solution (second washing) (10μ1)

lane 5: purified sample (10μ1)

Paragraph 4 starting on page 55 was amended as follows:

Other methods than [Taqman]TAGMAN method for optically detecting progress of PCR may be employed. For example, there may be employed an [amplisensor system] AMPLISENSOR SYSTEM (produced by Funakoshi Co.) which utilizes fluorescence-related energy transfer, a method which utilizes intercalation of ethidium bromide or the like (see Japanese Unexamined Patent Publication No.184397/1993), a method which utilizes change in fluorescence polarization (see Japanese Unexamined Patent Publication No.23800/1995) or the like. As other means than optical detection methods, util[i]ization of MALDI mass spectrometric analysis may be contemplated. It is possible that after completion of a reaction, one lid of a reaction vessel is removed, and necessary pre-treatment such as addition of a matrix reagent for MALDI is conducted to precisely measure molecular weights of a number of trace PCR products, thereby precisely detecting point mutation or repetition number of triplet repeat.

IN THE CLAIMS:

Claim 34 was amended as follows:

34. (Six times amended) A process for conducting a PCR reaction in a minute droplet of an aqueous solution protected from evaporation comprising the steps of:
providing a plate [planar]substrate;
providing an oily liquid layer;
providing an aqueous solution immiscible with said oily liquid layer;
shooting a minute droplet by inkjet of said aqueous solution into said oily liquid layer to contact said plate [planar] substrate;
providing a covering in contact with said oily liquid layer;
wherein said oily liquid layer surrounds all surfaces of said minute droplet of said aqueous solution that are not in contact with said plate [planar]substrate;
providing to said minute droplet a reactant; and conducting a PCR reaction in said minute droplet with said reactant whereby said PCR reaction is protected from evaporation [is reduced].

ABSTRACT

In a PCR reaction,[For a chemical reaction or the like], an extremely minute amount of a solution introduced [projected] by ink-jet method is retained on a substrate for a long period of time without evaporation [thereof]. On a substrate [10], a layer [11] of a liquid, which is hardly miscible with an extremely minute amount of a solution (minute droplet) intended to be retained, is formed. In the liquid layer [11], a minute droplet[13] is held [with the minute droplet] in contact with a surface of the substrate. If [When] the minute droplet [13] is aqueous, the liquid layer [11] coated over the surface of the substrate may be oily.